16:34:01 OCA PAD INITIATION - PROJECT HEADER INFORMATION 05/23/88

Active

Project #: G-33-613
Center # : Q5384-1A0
Cost share #:
Center shr #:
Rev #: 0
OCA file #:
Work type : RES
Document : GRANT
Contract entity: GIT

Contract#: 5 R01 EY07006-2
Prime #:
Mod #:

Subprojects ? : N
Main project #:

Project unit: CHEM Unit code: 02.010.136
Project director(s):
YU N-T CHEM

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 880401 to 890331 (performance) 890331 (reports)

Sponsor amount	New this change	Total to date
Contract value	84,470.00	84,470.00
Funded	84,470.00	84,470.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: CLINICAL MONITOR OF DIABETIC LENSES BY FLUORESCENCE/RAMAN

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) :
Defense priority rating :
Equipment title vests with: Sponsor

ONR resident rep. is ACO (Y/N) N
supplemental sheet
GIT X

Administrative comments -
INITIATION. 2ND YEAR OF A CONTINUING GRANT APPROVED FOR SUPPORT FOR 4 YEARS

12 / 88

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Date 2/10/89

Project No. G-33-613 Center No. Q5384-1A0

Project Director N.T. Yu School/Lab Chemistry

Sponsor DHHS/PHS/NIH/NEI

Contract/Grant No. R 501 EX07006-2 GTRC ____ GIT XX

Prime Contract No. ____

Title Clinical Monitor of Diabetic Lenses by Fluorescence / Raman

Effective Completion Date 3/31/89 (Performance) 3/31/89 (Reports)

Closeout Actions Required:

____ None
_x_ Final Invoice or Copy of Last Invoice - SF269
____ Final Report of Inventions and/or Subcontracts
____ Government Property Inventory & Related Certificate
____ Classified Material Certificate
____ Release and Assignment
____ Other ____

Includes Subproject No(s). ____

Subproject Under Main Project No. ____

Continues Project No. G-33-611 Continued by Project No. No award yet

Distribution:

_x_ Project Director
_x_ Administrative Network
_x_ Accounting
_x_ Procurement/GTRI Supply Services
_x_ Research Property Management
___ Research Security Services

_x_ Reports Coordinator (OCA)
___ GTRC
_x_ Project File
_x_ Contract Support Division (OCA) (2)
___ Other ____

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER EY07006-03	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	04/01/88	01/20/89

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Clinical Monitor of Diabetic Lenses by Fluorescence/Raman
(SEE INSTRUCTIONS)

1. **The Plans for the Next Year of Support:** Prior to our instrument relocation, we will continue our current *in vitro* fluorescence measurements of human lenses using the donor eyes from the Atlanta Lions Eye Bank. The primary purpose is to calibrate the new instrument and to establish some experimental procedures for the clinical measurements. Once the system is moved to the Joslin Diabetes Center, we will start our study of the diabetic subjects recruited from among the patients attending the Joslin Eye Unit. We will also study the normal control lenses from volunteer subjects. The specific aims for the next year of support are (1) To determine the performance characteristics of the system. Parameters such as sensitivity, signal-to-noise and grating grooves density will be optimized. (2) To detect the changes in fluorescence line shape associated with normal aging and with diabetes mellitus. The spectral difference (subtraction) technique will be employed to reveal the subtle line shape changes. (3) To initiate the search for a reliable procedure (or methodology) for predicting the onset of cataract formation.

2. **Concise Description of the Studies Conducted during the Current Budget Year :**

INSTRUMENTATION

The instrumentation fabrication has been completed and initial testing is currently being performed prior to relocating the system to the Joslin Diabetes Center for clinical measurements. A schematic of the instrumentation is illustrated in Fig. 1. The beam from a laser is steered through a compensated attenuator (A) and focused onto the input end of a 140 μm diameter optical fiber using a 40X microscope objective (L1). The attenuator

is used to modulate the laser power so that the intensity incident on the cornea is within acceptable clinical limits. The optical fiber delivers the excitation light to the illumination arm of the modified slit lamp. The illumination arm incorporates micrometer adjustments in height and rotation to facilitate positioning of the focal spot in the lens with respect to the observation optical system. The beam output from the optical fiber is focused to a 40 μm diameter spot in the lens of the eye using a 18 mm focal length orthoscopic lens system (L2).

The light emitted from the focal region in the lens of the eye is collected using a wide aperture (5 cm diameter) 6.2 cm focal length lens (L3), and focused onto the input aperture of a 450 μm diameter optical fiber positioned at the image plane of the observation eyepiece (E). The image of the illumination optical section in the lens of the eye can be observed directly through this eyepiece. The focal point of the optical section is positioned using the illumination arm adjustments so that the optical fiber aperture is coincident with the position of the image of the focal spot in the optical section. The light from this focal region collected through the collecting optical fiber aperture is delivered to the entrance slit of the spectrometer using a specially configured optical fiber bundle. The fiber bundle is comprised of individual 100 μm diameter fibers with a numerical aperture of 0.2. The output end of this fiber bundle is configured as a line slit 7 mm high by 100 μm wide. The output from the fiber bundle is magnified by a factor of 2 using lens system L4 and focused onto the entrance slit (S) of the spectrometer (M). This allows a matching of the numerical aperture of the spectrometer to the light incident on the entrance slit which optimizes the utilization of the available collected light from the lens of the eye.

The spectrum of the light incident on the spectrometer is dispersed using a single monochromator (M). The single monochromator was chosen as the throughput was greater than the originally proposed triple monochromator. Comparisons of the signals measured from

a fluorescence glass standard using these two spectrometers was performed. It was found that for a given incident laser power, detector gain, exposure time and spectral dispersion that the fluorescence intensity measured at 533.5 nm using the single monochromator was 2.6 times greater than that using the triple monochromator. In order to obtain grating ghost-free dispersion in the single monochromator, however, a holographic grating will have to be used in place of the current classically ruled grating. The holographic grating has been ordered and will be inserted as soon as it is available.

The dispersed spectrum of the light emitted from the lens is focused onto the face of an intensified 1024-element array detector (I) which is part of the Tracor Northern detection system (D). The resulting spectra are read off the detector and stored in the Tracor Northern analysis system for subsequent investigation.

IN VITRO LENS MEASUREMENTS

Lens measurements have been performed using the current instrumentation employing a Helium/Cadmium laser to provide excitation illumination at 441.6 nm. Measurements have been made from two whole excised lenses, a 42-year old lens and a 77-year old lens. The younger lens exhibited developing cortical opacities and minimal yellowing in the lens nucleus. The older lens exhibited significant yellow/brown sclerosis development in the nucleus.

The excitation laser power was adjusted so that 0.8 mW was incident on the front of the lens sample. The intensifier gain was set and maintained constant for the duration of the measurements. The exposure time for signal integration was set to 5 seconds. These parameters were well within safety guidelines for laser exposure to the retina and would be comparable to setting used for clinical measurements. For both lenses measurements were performed at 7 locations along the anterior/posterior lens axis. The results from these lenses are illustrated in Figs. 2 and 3.

Internal normalization of the fluorescence signal in this situation was performed using the amplitude of the unshifted laser line at 441.6 nm. This provides a measure of the Rayleigh scattering component from the lens. The reason the Rayleigh line was used here for normalization was that at this excitation wavelength any Raman signals are obscured by the large lens fluorescence signal. Figs. 2 and 3 illustrate both the scattering corrected fluorescence intensity profiles and the uncorrected intensity profiles. The effect of increased attenuation due to scattering can be appreciated by comparing the corrected and uncorrected fluorescence intensity profiles.

During the course of making measurements from these lenses it was noted that at a given position in the excised lens that the fluorescence intensity decreased with increasing exposure time. This indicated that the fluorophor giving rise to the measured fluorescence was becoming bleached by the incident illumination. The results of such a measurement from the 42-year old lens are illustrated in Fig. 4. The fluorescence intensity was measured continuously, from the same position in the lens, over a period of 20 minutes. The figure illustrates a plot of fluorescence intensity decrease versus time and the solid line is a single exponential fit to the data. The value of the exponent was used to calculate a half time of 5.6 minutes for this bleaching process. While the bleaching process is long compared to the exposure times to be used to measure lens fluorescence it should nevertheless be accounted for during measurements made at this excitation wavelength. Measurements also showed that over periods of 60 minutes that this bleaching process was not reversible and that if a partial bleaching was initiated followed by a period of 30 minutes in the dark, that the fluorescence intensity remained the same as that measured prior to dark adaptation and further bleaching continued from this point. This bleaching process and the dynamics of the process will be investigated further using the 441.6 nm excitation wavelength and at other excitation wavelengths.

3. No Change

4. Not Applicable

5. **Publications :**

(a) Bursell, Sven-Erik and Yu, Nai-Teng (1989) "Fluorescence and Raman Spectroscopy of the Crystallin Lens" in *New Developments in Noninvasive Studies to Evaluate Ocular Function* (Masters, B. R., Ed.) Springer-Verlag, New York (in press).

(b) Yu, Nai-Teng, Cai, M.-Z., Ho, D. J.-Y. and Kuck, J. F. R., Jr. (1988) "Automated Laser-Scanning-Microbeam Fluorescence/Raman Image Analysis of Human Lens with Multichannel Detection : Evidence for Metabolic Production of a Green Fluorophor" *Proc. Natl. Acad. Sci. USA*, *85*, 103-106.

(c) Yu, Nai-Teng, Barron, B. C. and Kuck, J. F. R., Jr. (1989) "Distribution of Two Metabolically Related Fluorophors in Human Lens Measured by Laser Microprobe" *Exp. Eye Res.* (in press).

(d) Yu, Nai-Teng Yu and Bursell, Sven-Erik (1988) "A New Approach to Study Human Cataractogenesis : Fluorescence/Raman Intensity Ratio and Imaging" in Spectroscopic and Structural Studies of Biomaterials I : Proteins (Twardowski, J., Ed.) Sigma Press, Wilmslow, Cheshire, U.K., pp.65-76.